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RESEARCH ARTICLE

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Labiobaetis Novikova & Kluge in Ethiopia (Ephemeroptera, Baetidae), with description of a new species

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Abstract

Material collected between 2017 and 2019 in Ethiopia in the Awash River catchment substantially increased our knowledge of *Labiobaetis* Novikova & Kluge in this country. Four species were previously reported based on ecological investigations of Ethiopian rivers: *L. glaucus* (Agnew, 1961), *L. latus* (Agnew, 1961), *L. vinosus* (Barnard, 1932) and *L. bellus* (Barnard, 1932). We have identified six different species using a combination of morphology and genetic distance (COI, Kimura 2-parameter). Two of them, *L. alahmadii* Gattolliat & Al Dhafer, 2018 and *L. potamoticus* Gattolliat & Al Dhafer, 2018 were previously assumed to be endemic to the Arabian Peninsula. The status of *L. bellus* is discussed and remains unresolved. One species is new to science; it is described and illustrated based on its nymphs. A key to the nymphs of all Ethiopian species is provided. The interspecific K2P distances in Ethiopia are between 17% and 23%, the intraspecific distances are usually between 0% and 1%. The total number of *Labiobaetis* species worldwide is augmented to 145. The Afrotropical species of *Labiobaetis* are discussed in comparison to the species of other realms.

Keywords

COI, genetic distance, integrative taxonomy, morphology

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Introduction

The family Baetidae has the highest species diversity among mayflies, comprising ca. 1,100 species in 114 genera (updated from Sartori and Brittain 2015; Jacobus et al. 2019; Cruz et al. 2020), which is approximately one third of all mayfly species worldwide. They have a cosmopolitan distribution except in New Zealand (Gattolliat and Nieto 2009). Investigations of the molecular phylogeny of the Order Ephemeroptera revealed the relatively primitive status of the family (Ogden and Whiting 2005; Ogden et al. 2009; Ogden et al. 2019).

Labiobaetis Novikova & Kluge, 1987, is one of the richest genera of mayflies with 144 previously described species (Barber-James et al. 2013; Kaltenbach et al. 2020 and citations therein). The distribution of *Labiobaetis* is nearly worldwide, except for the Neotropical realm, New Zealand and some remote islands. After a long period of controversy, *Labiobaetis* is nowadays widely accepted as a valid genus (Gattolliat 2001; Fujitani et al. 2003; Fujitani 2008; McCafferty et al. 2010; Kluge and Novikova 2011, 2014, 2016; Kluge 2012; Webb 2013; Kubendran et al. 2014, 2015; Shi and Tong 2014). The history and concept of the genus *Labiobaetis* were recently summarized in detail (Shi and Tong 2014; Kaltenbach and Gattolliat 2018). Kluge and Novikova (2016) established a new tribe Labiobaetini including the genera *Labiobaetis* and *Pseudopannota* Waltz & McCafferty, 1987, based on a unique combination of imaginal and nymphal characters.

Recently, integrative taxonomy was applied to collections from the highly diverse regions of Southeast Asia and New Guinea, where 65 species were described and named (Kaltenbach and Gattolliat 2018, 2019, 2020; Kaltenbach et al. 2020). This contribution will focus on the Afrotropical country of Ethiopia.

Taxonomic studies of *Labiobaetis* have a long history in the Afrotropical realm. First, several species were described from South Africa by Barnard (1932), Crass (1947) and Agnew (1961) under the genus *Baetis* Leach, 1815. Thereafter, Kopelke (1980) named a few species from Central Africa under *Baetis*, based on adults only. Later, Gillies (1993, 1994) published new species from West and East Africa, still assigned to *Baetis*. Lugo-Ortiz and McCafferty (1997) made a revision of *Labiobaetis* in the Afrotropical region including Madagascar and subsequently, Lugo-Ortiz et al. (2000) provided a revision of the widespread species *L. glaucus* (Agnew, 1961). Gattolliat (2001) described six new species in his comprehensive study of the genus *Labiobaetis* in Madagascar. Kluge and Novikova (2016) contributed to the fauna of Central Africa and defined the tribe Labiobaetini. Finally, Gattolliat et al. (2018) studied the species from Saudi Arabia, which is bordering the Palaearctic realm, and described two new species. Until now, *Labiobaetis* encompasses 25 species in the Afrotropical realm, including two species only known from Saudi Arabia (Barber-James et al. 2013; Gattolliat et al. 2018).

The examined material was collected between 2017 and 2019 during ecological studies of the Awash River (Englmaier et al. 2020; Kebede et al. 2020). The collection area encompassed the whole Awash River catchment, including its major affluents (Fig. 1). The Awash River is endorheic; it springs in the Ethiopian Highlands at an

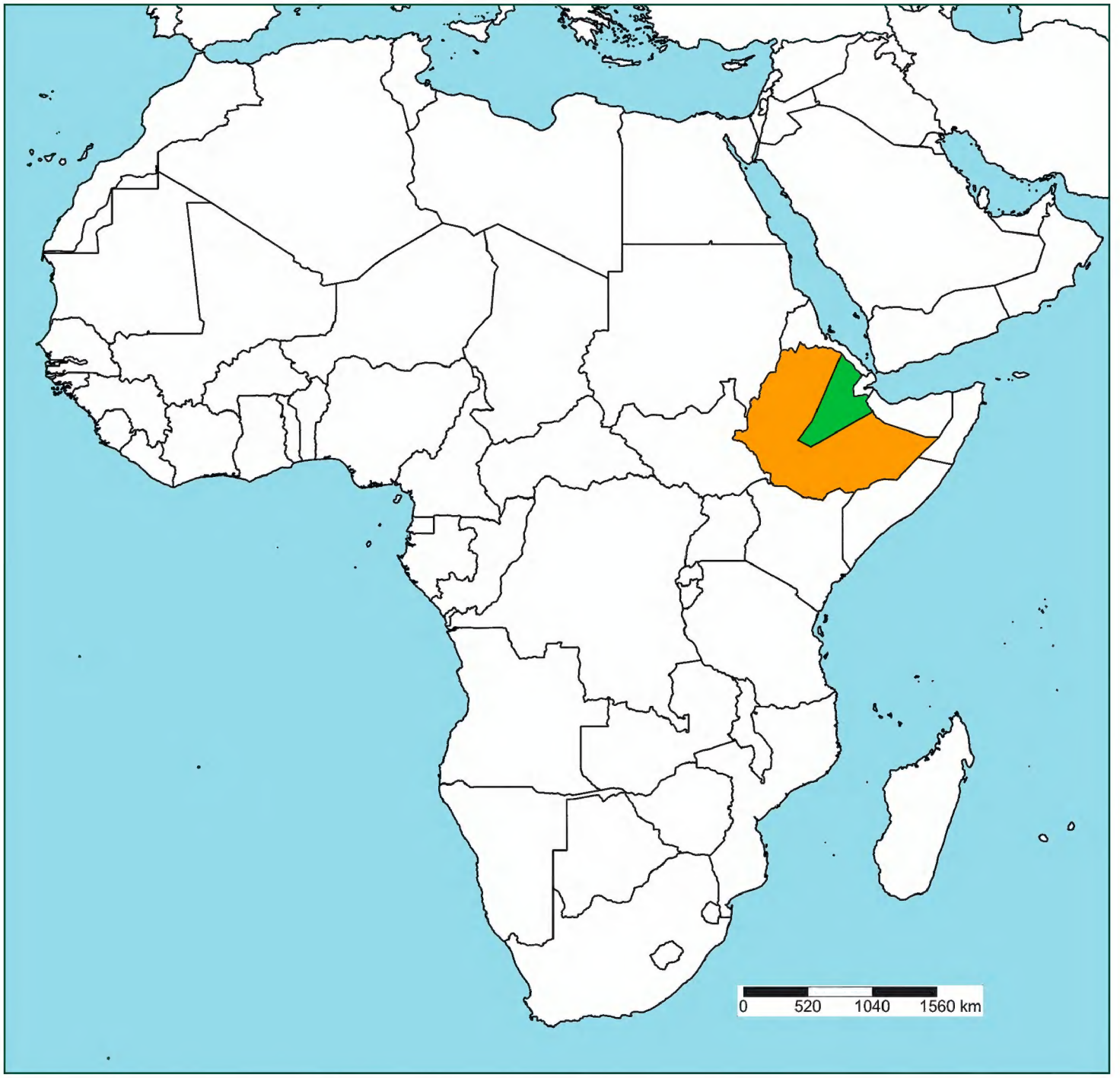

Figure 1. Map of Africa with Ethiopia (orange) including the Awash River catchment (green).

altitude of > 3000 m in the Chilimo Forest and flows into the arid Afar Depression, where it finally drains into the saline Lake Abbe at the Ethiopian-Djibouti border, at an altitude of ca. 250 m (Englmaier et al. 2020 and citations therein). The study area including the physical conditions at the sampling sites are described and illustrated in detail in Englmaier et al. (2020: fig. 1, table 1). Apart from the protected Chilimo Forest, the region is subject to extensive anthropogenic impact (intensive agriculture, overgrazing by livestock), resulting in the loss of natural vegetation (Englmaier et al. 2020 and citations therein). The eco-geographical features of Ethiopia, including altitude, geology, hydrology, rainfall, temperature, soil types and land cover, as well as its freshwater ecoregions, are described in Haile and Moog (2016). Ethiopia shares two ecoregions, mainly the Central Eastern Africa ecoregion, but also to a small extent the North Africa and Sahara Desert ecoregion in the northwestern part of the country (Barber-James and Gattolliat 2012).

Table 1. Sequenced specimens.

Species	Locality	Specimens catalog #	GenBank # (COI)	GenSeq Nomenclature
L. alahmadii	Ethiopia	GBIFCH00763723	MW307223	genseq-4 COI
		GBIFCH00763718	MW307225	genseq-4 COI
		GBIFCH00763720	MW307222	genseq-4 COI
		GBIFCH00763724	MW307224	genseq-4 COI
		GBIFCH00763732	MW307227	genseq-4 COI
		GBIFCH00763719	MW307226	genseq-4 COI
	Saudi Arabia	GBIFCH00517527	MH070307	genseq-2 COI
		GBIFCH00235747	MH070313	genseq-2 COI
		GBIFCH00235757	MH070314	genseq-2 COI
		GBIFCH00517526	MH070322	genseq-2 COI
		GBIFCH00465155	MH070291	genseq-2 COI
L. excavatus sp. nov.	Ethiopia	GBIFCH00763725	MW307229	genseq-2 COI
		GBIFCH00674636	MW307228	genseq-2 COI
L. glaucus	Ethiopia	GBIFCH00763728	MW307230	genseq-4 COI
	Saudi Arabia	GBIFCH00465151	MH070288	genseq-4 COI
		GBIFCH00235741	MH070311	genseq-4 COI
		GBIFCH00235750	MH105068	genseq-4 COI
		GBIFCH00235731	MH070317	genseq-4 COI
		GBIFCH00517523	MH070320	genseq-4 COI
	South Africa	GBIFCH00517537	MH070310	genseq-4 COI
		GBIFCH00517539	MH070321	genseq-4 COI
		GBIFCH00517538	MH070319	genseq-4 COI
	Mayotte	GBIFCH00517531	MH105069	genseq-4 COI
		GBIFCH00521580	MH070315	genseq-4 COI
		GBIFCH00517530	MH070318	genseq-4 COI
L. latus	Ethiopia	GBIFCH00763729	MW307231	genseq-4 COI
L. potamoticus	Ethiopia	GBIFCH00763731	MW307235	genseq-4 COI
		GBIFCH00763721	MW307233	genseq-4 COI
		GBIFCH00763727	MW307234	genseq-4 COI
		GBIFCH00674637	MW307232	genseq-4 COI
	Saudi Arabia	GBIFCH00517520	MH070306	genseq-2 COI
		GBIFCH00517521	MH070308	genseq-2 COI
		GBIFCH00235735	MH070312	genseq-2 COI
		GBIFCH00235732	MH070316	genseq-2 COI
		GBIFCH00465152	MH070289	genseq-2 COI
		GBIFCH00465154	MH070290	genseq-2 COI

So far, the diversity of *Labiobaetis* in Ethiopia has only become known through an ecological study of the benthic fauna of mountain streams and rivers (Harrison and Hynes 1988). Four species were reported in this study: *L. glaucus* (Agnew, 1961), *L. latus* (Agnew, 1961), *L. vinosus* (Barnard, 1932) and *L. bellus* (Barnard, 1932). The identity and status of *L. bellus* is unclear and will be discussed below. Here, we report three additional species from the Awash River catchment, one of which is described and illustrated as a new species, based on nymphs. The total number of *Labiobaetis* species worldwide is augmented to 145.

Materials and methods

All specimens were collected between 2017 and 2019 by Wolfram Graf (University of Natural Resources and Life Sciences, Austria) and Yonas Terefe (Ambo University, Ethiopia) and preserved in 70–96% ethanol.

Table 2. GPS coordinates of locations of examined specimens.

Species	Locality	GPS coordinates
L. alahmadii	Ethiopia: Mille River	11°24'50"N, 40°45'38"E
	Ethiopia: Korkada	08°30'03"N, 39°33'07"E
	Ethiopia: Lafessa	08°23'16"N, 38°54'31"E
	Ethiopia: Worer	09°20'07"N, 40°10'20"E
L. excavatus sp. nov.	Ethiopia: Awash	09°04'01"N, 38°08'09"E
L. glaucus	Ethiopia: Borkana River	10°39'59"N, 39°55'53"E
	Ethiopia: Lafessa	08°23'16"N, 38°54'31"E
	Ethiopia: Dubti	11°41'50"N, 41°07'23"E
	Ethiopia: Worer	09°20'07"N, 40°10'20"E
	Ethiopia: Sulula	08°39'57"N, 38°37'59"E
L. latus	Ethipoia: Lafessa	08°23'16"N, 38°54'31"E
L. potamoticus	Ethiopia: Dubti	11°41'50"N, 41°07'23"E
	Ethiopia: Worer	09°20'07"N, 40°10'20"E
	Ethiopia: Wonji	08°28'24"N, 39°12'44"E
	Ethiopia: Lafessa	08°23'16"N, 38°54'31"E
	Ethiopia: Awash Kunture	08°42'22"N, 38°36'19"E
	Ethiopia: Yimre	09°04'59"N, 40°10'03"E
L. vinosus	Ethiopia: Lafessa	08°23'16"N, 38°54'31"E
	Ethiopia: Korkada	08°30'03"N, 39°33'07"E

The dissection of nymphs was performed in Cellosolve (2-Ethoxyethanol) with subsequent mounting on slides with Euparal liquid, using an Olympus SZX7 stereomicroscope.

The DNA of part of the specimens was extracted using non-destructive methods allowing subsequent morphological analysis (see Vuataz et al. 2011 for details). We amplified a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using the primers LCO 1490 and HCO 2198 (Folmer et al. 1994; see Kaltenbach and Gattolliat 2020 for details). Sequencing was done with Sanger's method (Sanger et al. 1977). The genetic variability between specimens was estimated using Kimura-2-parameter distances (K2P, Kimura 1980), calculated with MEGA 7 (Kumar et al. 2016, http://www.megasoftware.net).

The GenBank accession numbers are given in Table 1, nomenclature of gene sequences follows Chakrabarty et al. (2013).

Drawings were made using an Olympus BX43 microscope. To facilitate the determination of the new species and the comparison of important structures with other species, we partly used a combination of dorsal and ventral aspects in one drawing (see Kaltenbach et al. 2020: fig. 1).

Photographs of nymphs were taken using a Canon EOS 6D camera and the Visionary Digital Passport imaging system (http://www.duninc.com) and processed with Adobe Photoshop Lightroom (http://www.adobe.com) and Helicon Focus version 5.3 (http://www.heliconsoft.com). Photographs were subsequently enhanced with Adobe Photoshop Elements 13.

The distribution maps were generated with SimpleMappr (https://simplemappr.net, Shorthouse 2010). The GPS coordinates of the sample locations are given in Table 2.

The dichotomous key was elaborated with the support of DKey version 1.3.0 (http://drawwing.org/dkey, Tofilski 2018).

The terminology follows Hubbard (1995) and Kluge (2004). The description follows the form of other recent descriptions of *Labiobaetis*, as for example in Kaltenbach et al. 2020.

Results

Abbreviations

MZL Musée de Zoologie Lausanne (Switzerland).

List of *Labiobaetis* species from Ethiopia

1. *L. alahmadii* Gattolliat & Al Dhafer, 2018
2. *L. excavatus* sp. nov.
3. *L. glaucus* (Agnew, 1961)
4. *L. latus* (Agnew, 1961)
5. *L. potamoticus* Gattolliat & Al Dhafer, 2018
6. *L. vinosus* (Barnard, 1932)
7. *L. bellus* (Barnard, 1932)

(*L. bellus*: unclear identity and status, no further treatment in this study, see discussion)

1. *Labiobaetis alahmadii* Gattolliat & Al Dhafer, 2018

Gattolliat et al. 2018: figs 20–33.

Differential diagnosis. ***Nymph.*** Following combination of characters: A) colouration: abdomen dorsally brown, with light pattern as Gattolliat et al. 2018: figs 32, 33; B) scape without distolateral process; C) labial palp segment II with thumb-like protuberance; segment III slightly pentagonal; D) maxillary palp segment II with excavation at inner distolateral margin; E) fore femur rather broad, length ca. 3× maximum width; dorsal margin with ca. 18 curved, spine-like setae and many fine, simple setae, and basally some additional spine-like setae near margin; femoral patch reduced; F) fore tibia dorsally with a row of short, spatulate setae (Gattolliat et al. 2018: fig. 26); G) hind protoptera well developed; H) seven pairs of gills; I) paraproct with ca. 16 stout, marginal spines.

Examined material. Ethiopia • 6 nymphs; Lower Mille River; 11°24'50"N, 40°45'38"E; 482 m; leg. W. Graf; 5 in alcohol; GenBank MW307224; GBIFCH00763724, GBIFCH00515555; 1 on slide; GenBank MW307223; GBIFCH00763723 • 1 nymph; Korkada; 08°30'03"N, 39°33'07"E; 09.12.2017; 1260 m; leg. W. Graf; Kk2; in alcohol; GenBank MW307225; GBIFCH00763718 • 1 nymph; Korkada; 08°30'03"N, 39°33'07"E; 1260 m; 09.11.2017; leg. W. Graf; Kk1; in alcohol; GenBank MW307226; GBIFCH00763719 • 1 nymph; Worer; 09°20'07"N,

40°10'20"E; 740 m; 29.01.2018; leg W. Graf; Wr1; in alcohol; GenBank MW307222; GBIFCH00763720 • 1 nymph; Lafessa; 08°23'16"N; 38°54'31"E; 1600 m; 05.11.2017; Lf1; leg. W. Graf; in alcohol; GenBank MW307227; GBIFCH00763732; all material in MZL.

Biological aspects. The specimens were collected at altitudes between 480 m and 1600 m. Further characteristics of sampling sites are given in Englmaier et al. 2020: table 1. In Saudi Arabia, the species occurs in medium-size streams with stony substrates, preferably in relatively fast flowing water or even at the base of small waterfalls (Gattolliat et al. 2018).

Distribution. Ethiopia (Fig. 2a), Saudi Arabia (Gattolliat et al. 2018).

2. *Labiobaetis excavatus* sp. nov.

http://zoobank.org/15AB1723-9D5C-4128-A058-719772F436D8

Figures 2a, 3–5

Differential diagnosis. ***Nymph.*** Following combination of characters: A) colouration: abdomen dorsally uniform brown; B) scape with well-developed distolateral process; C) labial palp segment II with broad, thumb-like distomedial protuberance; segment III oblong; D) maxillary palp segment II with strong excavation at inner distolateral margin; E) fore femur rather slender, length 3.6× maximum width; dorsal margin with 18–27 curved, spine-like setae, and a partial row of spine-like setae near margin; femoral patch absent; F) hind protoptera well developed; G) seven pairs of gills; H) paraproct with 15–20 stout marginal spines.

Description. ***Nymph*** (Figs 3–5). Body length 7.3–8.5 mm. Cerci: ca. 2/3 of body length. Paracercus: ca. 2/3 of cerci length. Antenna: approx. twice as long as head length.

Colouration (Fig. 3a, b). Head, thorax and abdomen dorsally brown, fore protoptera brown. Head, thorax and abdomen ventrally ecru, frons brown. Legs ecru, femora and tarsi apically brown. Caudalii brown.

Antenna (Fig. 4g) with scape and pedicel subcylindrical, with well-developed distolateral process at scape.

Labrum (Fig. 5a). Subrectangular, length 0.7× maximum width. Distal margin with medial emargination and small process. Dorsally with medium, fine, simple setae scattered over surface; submarginal arc of setae composed of one plus ca. 17 long, feathered setae. Ventrally with marginal row of setae composed of lateral and anterolateral long, feathered setae and medial long, bifid setae; ventral surface with ca. nine short, spine-like setae near lateral and anterolateral margin.

Right mandible (Fig. 5b, c). Incisor and kinetodontium fused. Incisor with four denticles; kinetodontium with three denticles, inner margin of innermost denticle with row of thin setae. Prostheca robust, apically denticulate. Margin between prostheca and mola slightly convex. Tuft of setae at apex of mola present.

Left mandible (Fig. 5d, e). Incisor and kinetodontium fused. Incisor with four denticles; kinetodontium with three denticles. Prostheca robust, apically with small

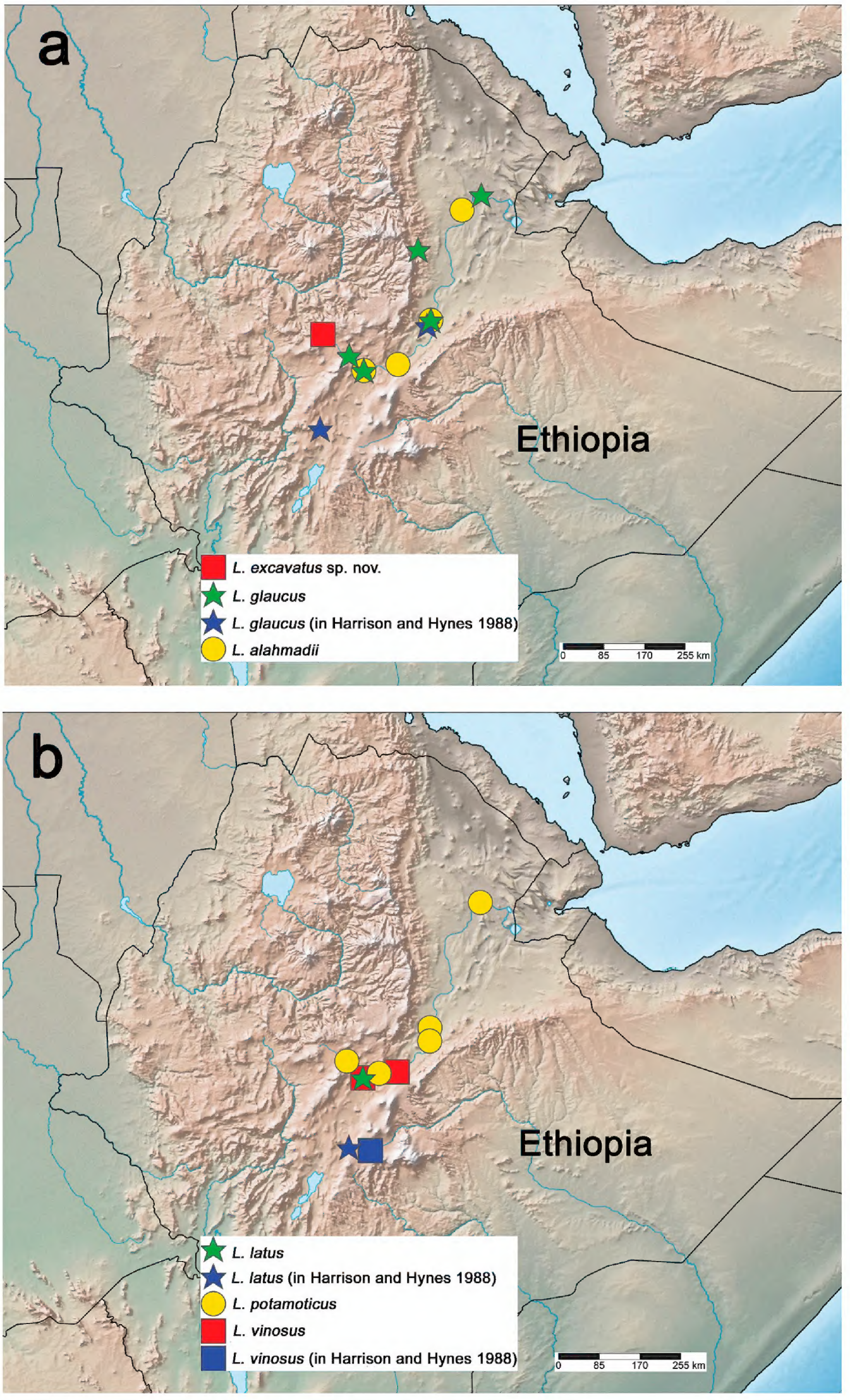

Figure 2. Distribution of *Labiobaetis* in Ethiopia.

Figure 3. *Labiobaetis excavatus* sp. nov., habitus, nymph **a** dorsal view **b** ventral view. Scale bars: 1.0 mm.

denticles and comb-shaped structure. Margin between prostheca and mola slightly convex, with minute denticles towards subtriangular process. Subtriangular process long and slender, above level of area between prostheca and mola. Denticles of mola apically constricted. Tuft of setae at apex of mola absent.

Both mandibles with lateral margins almost straight. Basal half with fine, simple setae scattered over dorsal surface.

Hypopharynx and superlinguae (Fig. 5f). Lingua longer than superlinguae. Lingua longer than broad; medial tuft of stout setae well developed, short; distal half laterally not expanded. Superlinguae distally rounded; lateral margins rounded; fine, long, simple setae along distal margin.

Maxilla (Fig. 5g, h). Galea-lacinia ventrally with two simple, apical setae under canines. Inner dorsal row of setae with three denti-setae, distal denti-seta tooth-like, middle and proximal denti-setae slender, bifid and pectinate. Medially with one pectinate, spine-like seta and six simple setae increasing in length distally. Maxillary palp slightly longer than length of galea-lacinia; 2-segmented; palp segment II 1.4× length of segment I; setae on maxillary palp fine, simple, scattered over surface of segments I and II; apex of last segment rounded, with strong excavation at inner distolateral margin.

Labium (Fig. 5i, j). Glossa basally broad, narrowing toward apex; shorter than paraglossa; inner margin with ca. seven spine-like setae, distalmost seta much longer

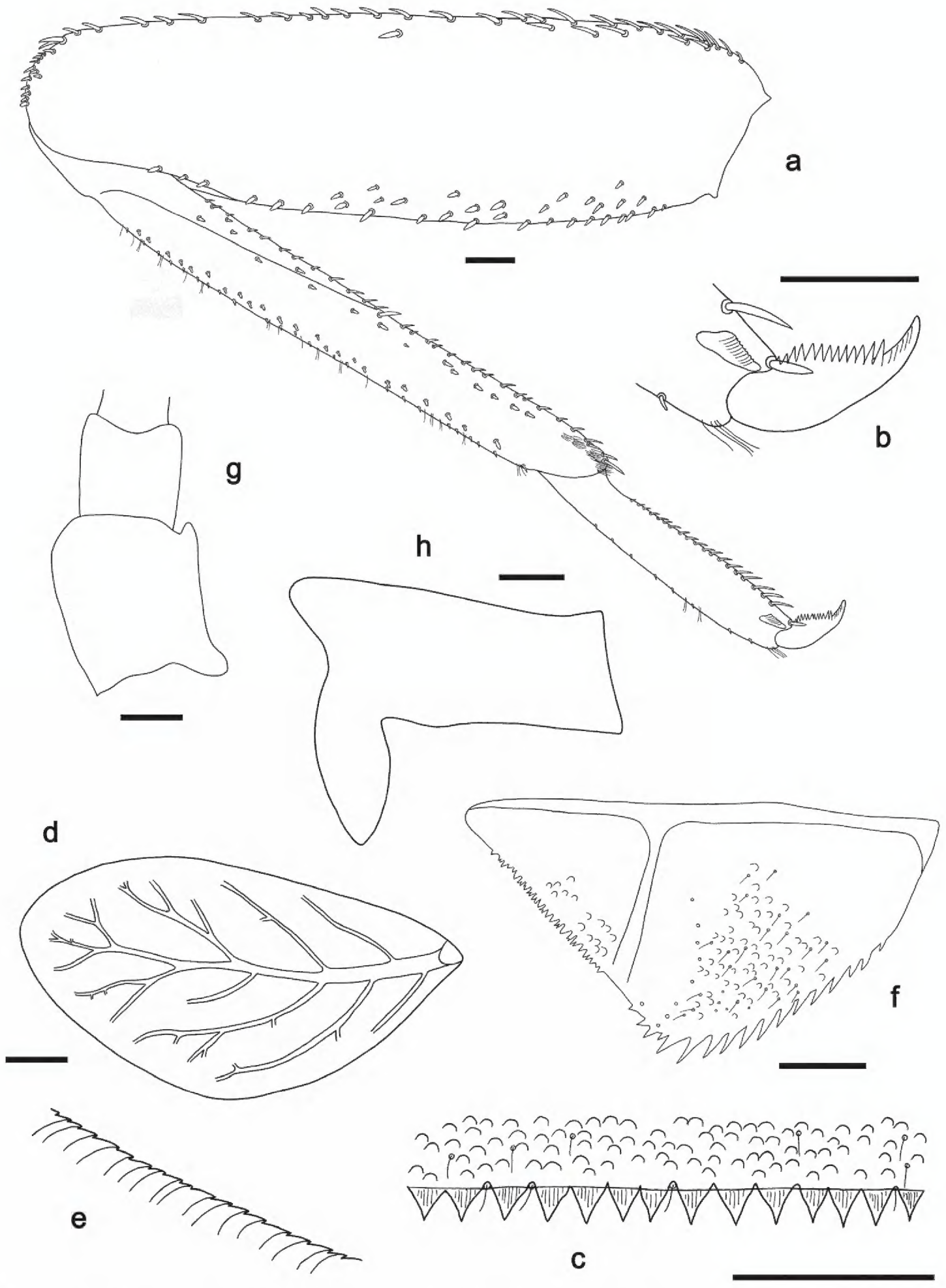

Figure 4. *Labiobaetis excavatus* sp. nov., nymph morphology **a** foreleg **b** fore claw **c** tergum IV **d** gill IV **e** margin of gill IV **f** paraproct **g** antennal scape **h** metanotum. Scale bars: 0.1 mm.

than other setae; apex with one long, one medium and one short, robust seta; outer margin with 5–7 spine-like setae increasing in length distally; ventral surface with fine, simple, scattered setae. Paraglossa sub-rectangular, curved inward; apex rounded;

with three rows of long, robust, distally pectinate setae in apical area and three or four medium, simple setae in anteromedial area; dorsally with row of five long, spine-like, simple setae near inner margin. Labial palp with segment I 0.7× length of segments II and III combined. Segment I ventrally with short, fine, simple setae. Segment II with broad thumb-like distomedial protuberance; distomedial protuberance 0.9× width of base of segment III; ventral surface with short, fine, simple setae; dorsally with two or three long, spine-like setae near outer margin. Segment III oblong; apex slightly pointed; length 1.2× width; ventrally covered with short, spine-like, simple setae and short, fine, simple setae.

Hind protoptera (Fig. 4h) well developed.

Foreleg (Fig. 4a, b). Ratio of foreleg segments 1.1:1.0:0.4:0.1. *Femur*. Length 3.6× maximum width. Dorsal margin with 18–27 curved, spine-like setae and partial second row near margin in basal area; length of setae 0.14× maximum width of femur. Apex rounded, with pair of spine-like setae and some short, stout setae. Many stout, lanceolate setae scattered along ventral margin; femoral patch absent. *Tibia*. Dorsal margin with row of short, stout setae and fine simple setae, and row of short, stout setae near margin. Ventral margin with row of short, curved, spine-like setae, distally of patellotibial suture one longer, curved, spine-like seta, on apex some longer setae and tuft of fine, simple setae. Anterior surface scattered with stout, lanceolate setae. Patellotibial suture present on basal half area. *Tarsus*. Dorsal margin with row of short, stout setae and fine, simple setae. Ventral margin with row of curved, spine-like setae. Claw with one row of 10–13 denticles; distally pointed; with ca. five stripes; subapical setae absent.

Terga (Fig. 4c). Surface with irregular rows of U-shaped scale bases and scattered fine, simple setae. Posterior margin of tergum IV with triangular spines, ca. as long as wide.

Gills (Fig. 4d, e). Present on segments I–VII. Margin with small denticles intercalating fine simple setae. Tracheae extending from main trunk to inner and outer margins. Gill I ca. 2/3 length of segment II; gill IV as long as length of segments V and half VI combined; gill VII slightly longer than length of segment VIII.

Paraproct (Fig. 4f). Distally not expanded, with 15–20 stout, marginal spines. Surface scattered with U-shaped scale bases, fine, simple setae and micropores. Cercotractor with small, marginal spines, partly split at apex.

Etymology. Referring to the strongly developed excavation at inner, distolateral margin of maxillary palp segment II.

Biological aspects. The specimens were collected at an altitude of 2400 m in relatively cold water (15.9 °C; see Englmaier et al. 2020: table 1). The sampling site lies in a protected area (S1, National Forest Priority Area), unlike all other sampling sites in this study (Englmaier et al. 2020).

Distribution. Ethiopia (Fig. 2a).

Type-material. ***Holotype.*** Ethiopia • nymph; Upper Awash River, Chilimo Forest; 09°04'01"N, 38°08'09"E; 2390 m; 06.11.2017; leg. W. Graf; on slide; GBIFCH00592380; MZL. ***Paratypes.*** Ethiopia • 9 nymphs; same data as holotype; 4 on slides; GenBank MW307229, MW307228; GBIFCH00763725, GBIFCH00674636, GBIFCH00592390, GBIFCH00592423; MZL; 5 in alcohol; GBIFCH00515502, GBIFCH00515552; MZL.

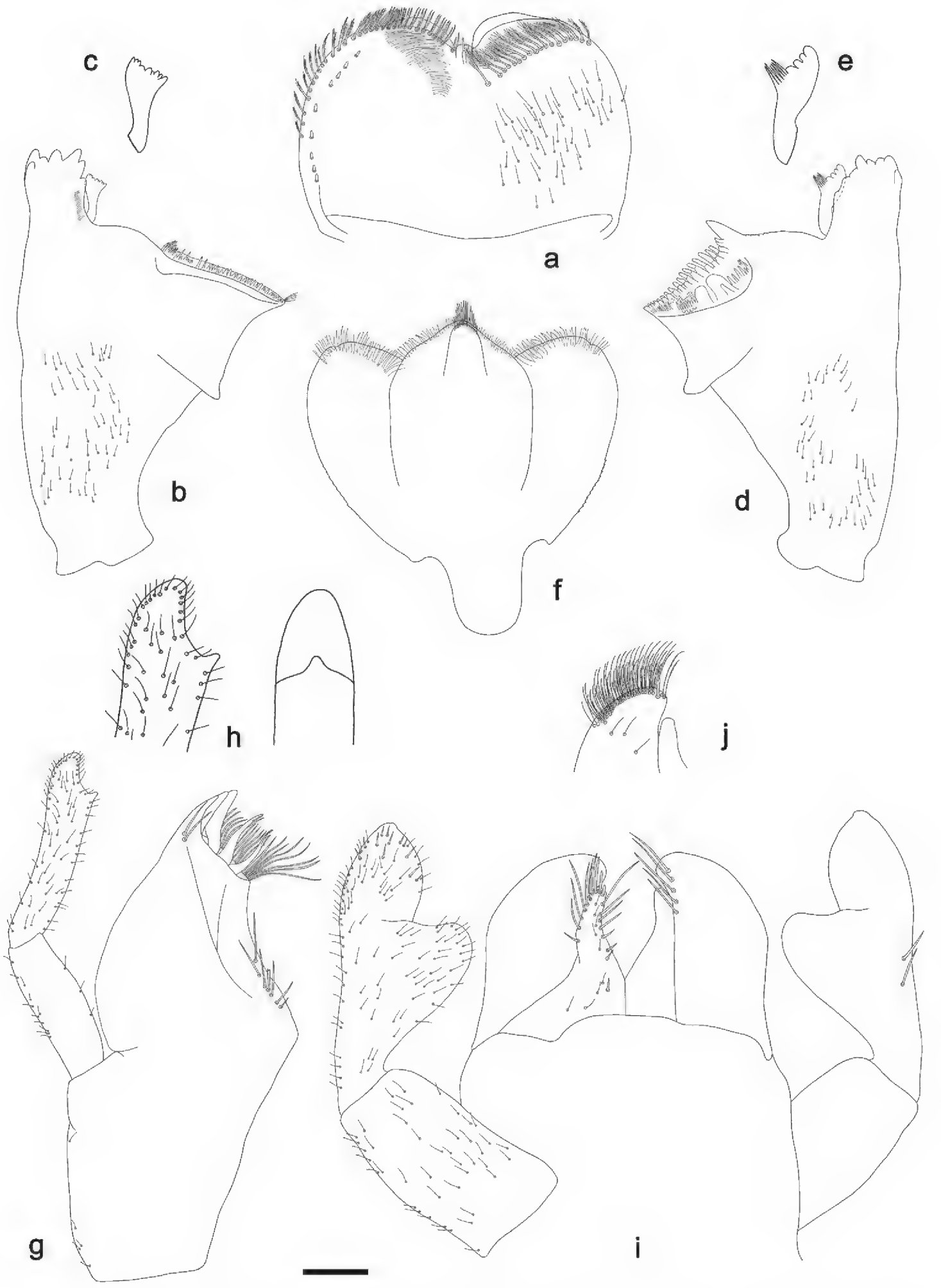

Figure 5. *Labiobaetis excavatus* sp. nov., nymph morphology: **a** labrum **b** right mandible **c** right prostheca **d** left mandible **e** left prostheca **f** hypopharynx and superlinguae **g** maxilla **h** apex of maxillary palp (left: dorsal view, right: inner lateral view) **i** labium **j** apex of paraglossa. Scale bar: 0.1 mm.

3. *Labiobaetis glaucus* (Agnew, 1961)

Agnew 1961 (*Baetis glaucus*)
Lugo-Ortiz and McCafferty 1997: figs 27–38, 39–50 (*Labiobaetis masai*, *L. nadineae*; both formal synonyms, Lugo-Ortiz et al. 2000)
Lugo-Ortiz et al. 2000 (*Pseudocloeon glaucum*)
Gattolliat et al. 2018: figs 34–44, 47

Differential diagnosis. ***Nymph.*** Following combination of characters: A) colouration: abdomen dorsally brown, with pattern as Gattolliat et al. 2018: fig. 47; B) scape without distolateral process; C) labial palp segment II with broad thumb-like protuberance; D) maxillary palp segment II with excavation at inner distolateral margin; E) fore femur rather broad, length ca. 3× maximum width; dorsal margin with 13–18 curved, spine-like setae and basally some additional setae near margin; femoral patch well developed; F) fore tibia dorsally with a row of scarce, tiny, stout setae (Gattolliat et al. 2018: fig. 40); G) hind protoptera well developed; H) seven pairs of gills; I) paraproct with 5–10 stout, marginal spines.

Examined material. Ethiopia • 6 nymphs; Middle Borkana River; 10°38'09"N, 39°55'53"E; 17.03.2019; 1413 m; leg. W. Graf; 1 on slide; GenBank MW307230; GBIFCH00763728; 5 in alcohol; GBIFCH00515556 • 4 nymphs; Lafessa; 08°23'16"N, 38°54'31"E; 1600 m; 08.11.2017; leg. W. Graf; Lf1; in alcohol; GBIFCH00515557 • 1 nymph; Dubti; 11°41'50"N, 41°07'23"E; 2017; 374 m; leg. W. Graf; S14; in alcohol; GBIFCH00515564 • 1 nymph; Sulula; 08°39'57"N, 38°37'59"E; 1916 m; 07.11.2017; leg. W. Graf; Su1; in alcohol; GBIFCH00515563 • 2 nymphs; Worer; 09°20'6.98"; 40°10'19.50"; 740 m; 29.01.2018; leg. W. Graf; Wr1; 1 on slide; GBIFCH00592437; 1 in alcohol; GBIFCH00515565; all material in MZL.

Biological aspects. The specimens were collected at altitudes from 370 m to 1920 m. Further characteristics of sampling sites are given in Englmaier et al. (2020). Harrison and Hynes (1988) reported the species from 750 m to 1900 m in stony runs and torrents. In Saudi Arabia, the species occurs in small, very shallow streams with moderate current and a substrate mixed of sand, cobbles and rock (Gattolliat et al. 2018).

Distribution. Ethiopia (Fig. 2a; Harrison and Hynes 1988), Saudi Arabia, Comoros (Gattolliat et al. 2018), South Africa, Lesotho, Namibia, Kenya (Lugo-Ortiz et al. 2000), Zimbabwe (Harrison and Hynes 1988) and potentially Iran (Tahmasebi et al. 2020).

4. *Labiobaetis latus* (Agnew, 1961)

Agnew 1961 (*Baetis latus*)
Lugo-Ortiz and McCafferty 1997: figs 1–13 (*Labiobaetis aquacidus*; formal synonym, Lugo-Ortiz and de Moor 2000)

Differential diagnosis. ***Nymph.*** Following combination of characters: A) scape with well-developed distolateral process; C) labial palp segment II with broad thumb-like protuberance; D) maxillary palp segment II with excavation at inner distolateral margin; E) fore femur rather broad, length ca. 3× maximum width; dorsal margin with 13–18 curved, spine-like setae; femoral patch rudimentary or absent; F) hind protoptera well developed; G) seven pairs of gills; H) paraproct with 21–29 stout, marginal spines.

Examined material. Ethiopia • 4 nymphs; Lafessa; 08°23'16"N, 38°54'31"E; 1600 m; 08.11.2017; leg. W. Graf; Lf1; 2 on slides; GenBank MW307231; GBIFCH00763729, GBIFCH00592391; 2 in alcohol; GBIFCH00515558, GBIFCH00515553; all material in MZL.

Biological aspects. The specimens were collected at an altitude of 1600 m. Further characteristics of the sampling site are given in Englmaier et al. (2020). Harrison and Hynes (1988) reported the species at 1900 m in marginal vegetation.

Distribution. Ethiopia (Fig. 2b), South Africa, Kenya (Lugo-Ortiz and McCafferty 1997).

5. *Labiobaetis potamoticus* Gattolliat & Al Dhafer, 2018

Gattolliat et al. 2018: figs 1–15, 19

Differential diagnosis. ***Nymph.*** Following combination of characters: A) colouration: abdomen dorsally brown, with pattern as Gattolliat et al. 2018: fig. 19; B) scape without distolateral process; C) labial palp segment II with small, thumb-like protuberance; segment III slightly pentagonal; D) maxillary palp segment II without excavation at inner distolateral margin; E) fore femur rather broad, length ca. 3× maximum width; dorsal margin with ca. 8 curved, spine-like setae; femoral patch reduced; F) hind protoptera well developed; G) seven pairs of gills; H) paraproct with ca. 36 stout, marginal spines.

Examined material. Ethiopia • 2 nymphs; Wonji; 08°28'24"N, 39°12'44"E; 1550 m; 09.11.2017; leg. W. Graf; Wj1; 1 on slide; GenBank MW307235; GBIFCH00763731; 1 in alcohol; GenBank MW307232; GBIFCH00674637 • 9 nymphs; Dubti; 11°41'50"N, 41°07'23"E; 374 m; leg. W. Graf; S14; 8 in alcohol; GBIFCH00515559; 1 in alcohol; GenBank MW307234; GBIFCH00763727 • 9 nymphs; Worer; 09°20'07"N, 40°10'20"E; 740 m; 29.01.2018; leg. W. Graf; Wr1; 8 in alcohol; GBIFCH00515560; 1 in alcohol; GenBank MW307233; GBIFCH00763721 • 2 nymphs; Yimre; 09°04'59"N, 40°10'03"E; 797 m; leg. W. Graf; 1 on slide; GBIFCH00592436; 1 in alcohol; GBIFCH00515566 • 1 nymph; Awash Kunture; 08°42'22"N, 38°36'19"E; 2003 m; 07.11.2017; leg. W. Graf; Ak1; in alcohol; GBIFCH00515567 • 1 nymph; Lafessa; 08°23'16"N, 38°54'31"E; 1600 m; 09.11.2017; leg. W. Graf; Lf1; in alcohol; GBIFCH00515568; all material in MZL.

Biological aspects. The specimens were collected at altitudes from 370 m to 2000 m. Further characteristics of sampling sites are given in Englmaier et al. (2020). In Saudi Arabia, the species occurs in aquatic vegetation in still reaches of small to medium-sized streams with sandy substrate (Gattolliat et al. 2018).

Distribution. Ethiopia (Fig. 2b), Saudi Arabia (Gattolliat et al. 2018) and potentially Iran (Tahmasebi et al. 2020).

6. *Labiobaetis vinosus* (Barnard, 1932)

Barnard 1932
Kopelke 1980 (*Pseudocloeon tenuicrinitum*; informal synonym, Kluge 2020)
Gillies 1994: figs 16–26 (*Baetis spatulatus*; formal synonym, Kluge and Novikova 2016)
Lugo-Ortiz and McCafferty 1997: figs 75–86
Kluge and Novikova 2016: figs 113, 122–129, 132, 133 (*L. tenuicrinitus*; informal synonym, Kluge 2020)

Remark. Judging from the figures and description in Kluge and Novikova (2016), there is no morphological difference between *L. vinosus* and *L. tenuicrinitus*. Kluge (2020) also indicates the synonymy of both species. However, no formal synonymy has been established so far. As we have not seen material of *L. tenuicrinitus*, we are not in a position to formally synonymise both species. Further, the genetic barcode (COI) of both species remains unknown.

Differential diagnosis. ***Nymph.*** Following combination of characters: A) colouration: abdomen dorsally brown, with pattern as Kluge and Novikova 2016: fig. 113; B) scape without distolateral process; C) labial palp segment II with broad, thumb-like protuberance; segment III conical; D) maxillary palp segment II with excavation at inner distolateral margin; E) fore femur rather broad, length ca. 3× maximum width; dorsal margin with 8–18 curved, spine-like setae and basally a partial second row of setae; F) hind protoptera absent or minute; G) six pairs of gills.

Examined material. Ethiopia • 6 nymphs; Lafessa; 08°23'16"N, 38°54'31"E; 1600 m; 08.11.2017; leg. W. Graf; Lf1; 1 on slide; GBIFCH00592392; 5 in alcohol; GBIFCH00515562, GBIFCH00763730, GBIFCH00829883, GBIFCH00829884, GBIFCH00829885 • 4 nymphs; Korkada; 08°30'03"N, 39°33'07"E; 1260 m; 10.11.2017; leg. W. Graf; Kk1; 3 in alcohol; GBIFCH00515561; 1 on slide; GBIFCH00592388; all material in MZL.

Biological aspects. The specimens were collected at altitudes of 1260 m and 1600 m. Further characteristics of sampling sites are given in Englmaier et al (2020). Harrison and Hynes (1988) reported the species at 2500 m in marginal vegetation.

Distribution. Ethiopia (Fig. 2b), DR Congo (Kopelke 1980), Tanzania (Gillies 1994), Uganda (Kluge and Novikova 2016), South Africa (Lugo-Ortiz and McCafferty 1997).

Key to the *Labiobaetis* species of Ethiopia (nymphs; excluding *L. bellus*)

1	Six pairs of gills	***L. vinosus***
–	Seven pairs of gills	**2**
2	With distolateral process at scape	**3**
–	Without distolateral process at scape	**4**

3 Maxillary palp with a strongly developed distolateral excavation (Fig. 5g, h), femur dorsally with row of 18 to 27 spine-like setae on margin and a partial row near margin (Fig. 4a), paraproct with 15 to 20 marginal spines (Fig. 4f) ..... ***L. excavatus* sp. nov.**

– Maxillary palp with distolateral excavation, femur dorsally with a row of 13 to 18 spine-like setae on margin, paraproct with 21 to 29 marginal spines (Lugo-Ortiz and McCafferty 1997: figs 6, 8, 13) ..... ***L. latus***

4 Labial palp segment II with broad thumb-like distomedial protuberance (Gattolliat et al. 2018: figs 24, 39) **5**

– Labial palp segment II with narrow thumb-like distomedial protuberance (Gattolliat et al. 2018: fig. 8) ..... ***L. potamoticus***

5 Body dorsally with pattern as in Gattolliat et al. 2018: fig. 32, femoral patch poorly developed, tibia dorsally with row of short, spatulate setae (Gattolliat et al. 2018: fig. 26) ..... ***L. alahmadii***

– Body dorsally with pattern as in Gattolliat et al. 2018: fig. 47, femoral patch well developed, tibia dorsally with row of scarce, tiny, stout setae (Gattolliat et al. 2018: fig. 40) ..... ***L. glaucus***

Genetics

COI sequences were obtained for five species (Table 1); we failed to get a sequence of *L. vinosus*, despite several trials. The genetic distances (K2P) among the species are between 17% and 23%, and therefore much higher than 3.5%, which is generally considered as a likely maximal value for intraspecific divergence (Hebert et al. 2003; Ball et al. 2005; Zhou et al. 2010) (Table 3). Very limited genetic distances (between 0% and 4%) were found between specimens of the same species, as in *L. potamoticus*, *L. excavatus* sp. nov. and *L. alahmadii*.

Discussion

Assignment to *Labiobaetis* and affinities

For the assignment of the new species to *Labiobaetis* we refer to Kluge and Novikova (2014). *Labiobaetis* is characterized by a number of derived characters, some of which are not found in other taxa (Kluge and Novikova 2014): antennal scape sometimes with a distolateral process (Fig. 4g); maxillary palp two segmented with excavation at inner distolateral margin of segment II, excavation may be poorly developed or absent (Fig. 5g); labium with paraglossae widened and glossae diminished; labial palp segment II with distomedial protuberance (Fig. 5i). The concept of *Labiobaetis* is also based on additional characters, summarized and discussed in Kaltenbach and Gattolliat (2018, 2019). *Labiobaetis excavatus* sp. nov. is morphologically related to *L. latus*, sharing the distolateral process at scape, well-developed hind protoptera, seven pairs of gills, and the broad, distomedial protuberance at segment II of the labial palps. The

Table 3. Intraspecific (bold) and interspecific genetic distances of the sequenced specimens (COI; Kimura 2-parameter; %, mean, minimum-maximum).

	Species	Locations	1	2	3	4	5
1	*L. alahmadii*	Ethiopia, Saudi Arabia	**1**				
			0–4				
2	*L. excavatus* sp. nov.	Ethiopia	19	**1**			
			18–20				
3	*L. glaucus*	Ethiopia, Saudi Arabia, South Africa, Mayotte	19	22	**1**		
			18–20	21–23	**0–2**		
4	*L. latus*	Ethiopia	19	21	20	–	
			19–20	21	20–21		
5	*L. potamoticus*	Ethiopia, Saudi Arabia	18	20	19	18	**2**
			17–19	19–20	18–20	17–18	**0–4**

main differences are the stronger distolateral excavation at the maxillary palp of *L. excavatus* sp. nov. (Fig. 5g, h; Lugo-Ortiz and McCafferty 1997: fig. 6), the number of spine-like setae at dorsal margin of femur (18–27 in *L. excavatus* sp. nov., plus a partial second row near margin; 13–18 in *L. latus*) and the presence or absence of setae at the apex of the left mola (present in *L. latus*, absent in *L. excavatus* sp. nov.). The strong distolateral excavation of the maxillary palp is very similar to *L. punctatus* Gattolliat, 2001, from Madagascar, which is also missing the setae at apex of the mola of the left mandible. However, the Malagasy species has no distolateral process at scape and differs by many other characters (Gattolliat 2001: figs 44–54).

Comparison to other realms and species groups

Remarkably, all Afrotropical species of *Labiobaetis* have a submarginal arc of feathered setae on the dorsal surface of the labrum (Gillies 1994; Lugo-Ortiz et al. 1999; Gattolliat 2001; Gattolliat et al. 2018, this study). In contrast, several additional types of these setae were described from all other regions. The majority of species occur in the Oriental realm and New Guinea. In New Guinea, simple setae were the predominant type, but also feathered setae, clavate setae with pectination, dendritic and lanceolate setae with and without pectination were described (Lugo-Ortiz et al. 1999; Kaltenbach and Gattolliat 2018). In Southeast Asia, simple, feathered and clavate setae are predominant and comparably frequent, but also lanceolate and dendritic setae were described (Müller-Liebenau 1984; Shi and Tong 2014; Kaltenbach and Gattolliat 2019, 2020; Kaltenbach et al. 2020). The type of the dorsal, submarginal setae together with the shape of the distomedial protuberance of labial palp segment II and often combined with other characters are building the base for the morphological species groups defined in Southeast Asia and New Guinea (Kaltenbach and Gattolliat 2018, 2019; Kaltenbach et al. 2020). These morphological groups within *Labiobaetis* are primarily a working tool but some may be natural groups and could also serve as a basis for future studies on the generic delimitation and phylogeny of this genus. Afrotropical *Labiobaetis* are not only sharing the feathered type of dorsal, submarginal setae on the labrum, but also have mostly a broad thumb-like distomedial protuberance of labial palps segment II. A lot of the variation between the species is coming from different

combinations of characters like seven or six pairs of gills, presence or absence of hind protoptera and presence or absence of a distolateral process at scape. The reduction and secondary loss of these characters seems to be a general tendency in *Labiobaetis* (Kluge and Novikova 2014; Kaltenbach and Gattolliat 2018, 2019) and they are, therefore, less reliable characters to define morphological groups. There are a few species with a narrow distolateral protuberance at labial palps segment II (*L. piscis* Lugo-Ortiz & McCafferty, 1997; *L. longicercus* Gattolliat, 2001; *L. potamoticus*), which are at the same time sharing seven pairs of gills, the absence of a distolateral process at scape and, more important, the absence of setae at the apex of the mola of the left mandible. These species are probably forming a morphological group amongst the other Afrotropical species. However, this is out of the scope of this paper and further investigations on other Afrotropical regions are necessary to discuss possible relationships of *Labiobaetis* species in this realm. Based on the present knowledge, all Afrotropical species of *Labiobaetis* seem to be morphologically closely related to the Southeast Asian *operosus* and *difficilis* groups (Kaltenbach and Gattolliat 2019). Both groups are very close to each other; the only difference is the presence (*operosus* group) or absence (*difficilis* group) of hind protoptera, which is a rather unreliable group character (see above).

The distribution of the *Labiobaetis* species seems to be also different in the Afrotropical realm compared to Southeast Asia and New Guinea. Apart from Madagascar, where all *Labiobaetis* species are endemic to the island (Gattolliat 2001), some Afrotropical species have a wide or even very wide distribution, e.g. *L. potamoticus* (Saudi Arabia, Ethiopia, potentially Iran), *L. latus* (Ethiopia, Kenya, South Africa), *L. vinosus* (Ethiopia, DR Congo, Tanzania, Uganda, South Africa) and especially *L. glaucus* (Ethiopia, Iran (?), Saudi Arabia, Comoros, Kenya, Namibia, Zimbabwe, South Africa). On the contrary, most species in Southeast Asia and New Guinea are restricted to smaller regions or are endemic to one island. An exception is *L. moriharai* Müller-Liebenau, 1984, known from Malaysia, Vietnam and Borneo (Kaltenbach and Gattolliat 2018, 2019, 2020; Kaltenbach et al. 2020). The reason for this difference is probably due to the high number of islands in Southeast Asia, especially in Indonesia and the Philippines, and the extreme landscape structure in New Guinea, facilitating allopatric speciation and endemicity (Toussaint et al. 2013, 2014; Kaltenbach and Gattolliat 2018, 2019; Kaltenbach et al. 2020). The huge African continent is in comparison geographically less structured, which is generally facilitating larger distribution areas of species.

Labiobaetis bellus

Since its description as a new species by Barnard (1932), *L. bellus* was regularly reported from South Africa and other countries, mainly in ecological studies of rivers (e.g. Crass 1947; Harrison 1950; Kimmins 1960; Oliff and King 1964; Chutter 1970, 1971; Harrison and Hynes 1988; Samways et al. 2011). However, apart from a rather sketchy drawing of the labial palp (Barnard 1932: fig. 13k), there are no further drawings of the mouthparts in Barnard (1932) and his description of the nymph is not precise enough to differentiate it unambiguously from other spe-

cies. Additionally, he mentioned that *L. bellus* and *Cheleocloeon excisum* (Barnard, 1932) "...approach each other very closely in the character of the mouth-parts of the nymphs." (Barnard 1932: 204). Later, already Kimmins (1960) was not sure about his determination of "*Baetis* ? *bellus*" from Uganda and proposed to solve the determination issues by studying nymphs rather than adults. Lugo-Ortiz and McCafferty (1997) did not mention *L. bellus* at all in their comprehensive study on Afrotropical *Labiobaetis*, contrary to *L. vinosus*, which Barnard (1932) described in the same paper. We may assume that these authors could not clarify the identity and the status of *L. bellus*. It remains unclear what Harrison and Hynes (1988) and other authors include in their concept of "*L. bellus*". Moreover, most of the reports of the species were anterior to the revision of the genus in the Afrotropics (Lugo-Ortiz and McCafferty 1997) and must be therefore considered as uncertain. Therefore, we refrain from further treatment of *L. bellus* before its species concept is clarified based on material from South Africa.

In comparison to *L. excavatus* sp. nov. with its broad distomedial protuberance at labial palp segment II similar to *L. latus*, the drawing of *L. bellus* in Barnard 1932: fig. 13k shows a more slender protuberance, more similar to *L. piscis* and *L. potamoticus*; *Labiobaetis piscis* and *L. potamoticus* may be easily confused with each other and *L. potamoticus* is abundant in the Awash River. In addition, *L. bellus* was reported from several places and different altitudes in the Awash River, contrary to *L. excavatus* sp. nov., which was found in the natural Chilimo Forest (2400 m) only, despite intensive sampling efforts along the Awash River. Further, *L. excavatus* sp. nov. is very similar to *L. latus*, which is reported additionally to *L. bellus* by Harrison and Hynes (1988). Therefore, we may assume that "*L. bellus*" sensu Harrison and Hynes (1988) has obvious differences to *L. latus* and thus to *L. excavatus* sp. nov. as well. As a conclusion, we assume that *L. excavatus* sp. nov. cannot be conspecific with *L. bellus*, the latter species being in the need of a taxonomic revision.

Genetic distance

The interspecific genetic distances found in Ethiopia (17–23%, Table 3) are in line with the ones between *Labiobaetis* species in other regions like New Guinea (average 22%; Kaltenbach and Gattolliat 2018), Indonesia (11–24%; Kaltenbach and Gattolliat 2019), Borneo (19–25%; Kaltenbach and Gattolliat 2020) and the Philippines (15–27%; Kaltenbach et al. 2020). Ball et al. (2005) reported a mean interspecific, congeneric distance of 18% for mayflies from the United States and Canada.

Two species, *L. alahmadii* and *L. potamoticus*, have intraspecific distances of up to 4%. In *L. alahmadii*, two specimens from Ethiopia have of genetic distance of 3%–4% to all other sequenced specimens from Ethiopia and Saudi Arabia. All other specimens have distances of 0%–1% between themselves, as well in Ethiopia as between Ethiopia and Saudi Arabia. Intraspecific distances of 4%–6% were also reported in some cases for *Labiobaetis* species in New Guinea, Indonesia, Borneo and the Philippines (Kaltenbach and Gattolliat 2018, 2019, 2020; Kaltenbach et al. 2020), as well as in aquatic beetles in the Philippines (Komarek and Freitag 2020). Ball et al. (2005) also reported

a case with 6% intraspecific distance in a mayfly in North America and intraspecific K2P distances of more than 3.5% are not uncommon within Plecoptera as well (Gill et al. 2015; Gattolliat et al. 2016). In *L. potamoticus*, the specimens from Ethiopia have distances of 0–1% between each other, and the higher distances of 3–4% are only between specimens from Ethiopia and Saudi Arabia, which can be explained by the greater geographic distance.

The COI sequence of *L. latus* from Ethiopia has a distance of 22% to another specimen from South Africa, reported in Gattolliat et al. (2018: table 1; GenBank MH070297, GBIF00465142), without any morphological difference between the two specimens. In the meantime, a second specimen from the same location in South Africa was sequenced and has the same barcode as the first specimen. Further, several COI barcodes with a distance of just 5–6% to the one from Ethiopia were obtained from specimens in South Africa as well, which may be explained by the geographic distance between Ethiopia and South Africa. There seem to be two different widespread mitochondrial lineages corresponding to the morphological concept of *L. latus*. This problem cannot be solved without additional investigations, including in particular nuclear genes, as it was recently done in the similar case of *Baetis harrisoni* Barnard, 1932 (Pereira da Conceicoa et al. 2012). Different mitochondrial lineages with the same morphology were already reported several times in *Labiobaetis* (Kaltenbach and Gattolliat 2018, 2019; Kaltenbach et al. 2020).

The number of sampled localities and different habitats in Ethiopia is still limited and there are regions without any collection activities so far (Fig. 2). However, the distribution of *Labiobaetis* species in Africa is often much more widespread than in other regions and suitable habitats are limited in this semiarid area. Therefore, we may expect a few, but not many more species to be discovered in Ethiopia with further collections.

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